Chapter 20

Further Development of the N-Gas Mathematical Model

An Approach for Predicting the Toxic Potency of Complex Combustion Mixtures

Barbara C. Levin, Emil Braun, Magdalena Navarro, and Maya Paabo

National Institute of Standards and Technology, Gaithersburg, MD 20899

A methodology has been developed for predicting smoke toxicity based on the toxicological interactions of complex fire gas mixtures. This methodology consists of burning materials using a bench-scale method that simulates realistic fire conditions, measuring the concentrations of the following primary fire gases - CO, CO_2, O_2, HCN, HCl, HBr, and NO_2 - and predicting the toxicity of the smoke using an empirical mathematical model called the N-Gas Model. The model currently in use is based on toxicological studies of the first six of the above listed primary gases both as individual gases and complex mixtures. The predicted toxic potency (based on this N-Gas Model) is checked with a small number of animal (Fischer 344 male rats) tests to assure that an unanticipated toxic gas was not generated. The results indicate whether the smoke from a material or product is extremely toxic (based on mass consumed at the predicted toxic level) or unusually toxic (based on the gases deemed responsible). The predictions based on bench-scale laboratory tests have been verified with full-scale room burns of a limited number of materials of widely differing characteristics chosen to challenge the system. The advantages of this approach are: 1. The number of test animals is minimized by predicting the toxic potency from the chemical analysis of the smoke and only using a few animals to check the prediction; 2. Smoke may be produced under conditions that simulate the fire scenario of concern; 3. Fewer tests are needed, thereby reducing the overall cost of the testing; and 4. Information is obtained on both the toxic potency of the smoke and the responsible gases. These results have been used in computations of fire hazard, and this methodology is now part of a draft international standard that is currently being voted on by the member countries of the International Standards Organization (ISO), Technical Committee 92 (TC92). In this chapter, a new 7-Gas Model including NO_2 and the data used in its development are presented.

The majority of people that die from exposure to fires are primarily affected by inhaling the toxic gases present in the smoke and not from burns. Fire death statistics examined for the years 1979 to 1985 attributed two-thirds of the victims to smoke inhalation and only one-third to burns (*1*). During these years, total fire deaths decreased 17%, but this decrease is primarily due to 34% fewer burn victims. Smoke fatalities only dropped 6%. The share of total fire deaths attributed to smoke inhalation has actually increased approximately 1% per year during this period. To further decrease deaths due to fires, the emphasis needs to be placed on reducing the number of deaths due to smoke inhalation. To accomplish this goal, we need to know the toxic products that are generated when materials thermally decompose and the toxicological interactions of the gases found in the complex chemical mixtures created by fires.

Our objectives in the development of the N-Gas Model were :

- To establish the extent to which we can explain and predict the toxicity of a material's combustion products by the interaction of the major toxic gases generated when that material is thermally decomposed in the laboratory,

- To develop a bioanalytical screening test which examines whether a material produces extremely toxic or unusually toxic combustion products and a mathematical model which predicts that toxicity,

- To predict the occupant response from the concentrations of primary toxic gases present in the environment and the time of exposure, and

- To provide data for use in computer models to predict the hazard that people will experience under various fire scenarios.

The N-Gas model using 6 gases (see Equation 1) is based on the hypothesis that a small number ("N") of gases in the smoke accounts for a large percentage of the observed toxic potency (*2-9*). This equation is based on studies at NIST on the toxicological interactions of six gases, CO, CO_2, HCN, reduced O_2, HCl and HBr. The concentrations of each of these gases necessary to cause 50% of the laboratory test animals (Fischer 344 male rats) to die either during the exposure (within exposure LC_{50}) or during the exposure plus the 14 day post-exposure observation period (within plus post-exposure LC_{50}) was determined. (The studies on HCl and HBr were conducted at Southwest Research Institute under a grant from NIST.) Similar measurements for various combinations of these gases indicated the additive, synergistic, and antagonistic toxicological effects of these gases. In this chapter, we will present the new data on NO_2 and discuss its impact on the N-Gas Model.

The LC_{50} may also refer to the mass of material loaded in the furnace or consumed by the exposure divided by the animal exposure chamber volume (g/m^3) which causes 50% of the animals to die within exposure or within exposure plus post-exposure. To reduce the number of animals necessary to determine an LC_{50} value, the material's LC_{50} is predicted by the N-Gas model. When the mass of material burned generates sufficient gaseous combustion products (those in the model) to produce an N-

Gas Value approximately equal to 1, that mass of material should be close to the material's LC_{50}. One or two animal tests can be conducted to check the predicted value and assure that an unexpected toxic gas was not produced. In most of the studies presented here, the exposure time was 30 minutes. We have also determined the LC_{50}'s of many of these gases both singly and mixed at times ranging from 1 to 60 minutes and have found that in all the cases examined that Equation 1 holds if the LC_{50}'s for the other times are substituted into the equation (*5,6*).

The N-Gas model prediction for 6 gases is based on the following empirical mathematical relationship:

$$\text{N-Gas Value} = \frac{m[CO]}{[CO_2] - b} + \frac{[HCN]}{LC_{50}HCN} + \frac{21 - [O_2]}{21 - LC_{50}O_2} + \frac{[HCl]}{LC_{50}HCl} + \frac{[HBr]}{LC_{50}HBr} \quad (1)$$

where the numbers in brackets indicate the time-integrated average atmospheric concentrations during a 30 minute (or other time) exposure period [(ppm x min)/min or for O_2 (% x min)/min]. Although CO_2 at concentrations generated in fires would not be lethal (the 30 minute LC_{50} of CO_2 is 470,000 ppm or 47% and the highest level of CO_2 possible in a fire is 21% and that would only happen if all the O_2 were converted to CO_2 which is highly unlikely), we found a synergistic effect between CO_2 and CO such that as the concentration of CO_2 increases (up to 5%), the toxicity of CO increases. Above 5% CO_2, the toxicity of CO starts to revert back towards the toxicity of CO by itself. The terms m and b define this synergistic interaction and in the 30 minute exposures, m and b equal -18 and 122000 if the CO_2 concentrations are 5% or less. For 30 minute studies in which the CO_2 concentrations are above 5%, m and b equal 23 and -38600, respectively. We have also shown that carbon dioxide increases the toxicity of the other gases currently included in the model as well as NO_2 (*3,10*). However, we found empirically that the effect of the CO_2 can only be added into this equation once. We, therefore, included the CO_2 effect into the CO factor for the following reasons: (1) we have examined the effect of many different concentrations of CO_2 on the toxicity of CO and have only examined the effect of 5% CO_2 on the other gases (5% was chosen based on data that showed that 5% CO_2 caused the greatest increase in CO toxicity) and (2) CO is the toxicant most likely to be present in all real fires.

The LC_{50} value of HCN is 200 ppm for 30 minute exposures or 150 ppm for 30 minute exposures plus the post-exposure observation period. (Exposure to CO in air only produced deaths during the actual exposures and not in the post-exposure observation period, whereas, HCN did cause numerous deaths in the first 24 hours of the post-exposure period). The 30 minute LC_{50} of O_2 is 5.4% which is subtracted from the normal concentration of O_2 in air, i.e., 21%. The LC_{50} value of HCl or HBr, respectively, for 30 minute exposures plus post-exposures times is 3700 ppm (*11*) and

3000 ppm (Switzer, W.G., Southwest Research Institute, personal communication).

In our pure and mixed gas studies, we found that if the value of Equation 1 is approximately 1, then some fraction of the test animals would die. Below 0.8, no deaths would be expected and above 1.3, all the animals would be expected to die. Since the concentration-response curves for animal lethalities from smoke are very steep, it is assumed that if some percentage (not 0 or 100%) of animals die, the experimental loading is close to the predicted LC_{50} value. Our results using this method show good agreement (deaths of some of the animals when the N-gas values are above 0.8) and the good predictability of this approach.

This model can be used to predict deaths that will occur only during the smoke exposure or those that will occur during and following the exposure. (The animals were not treated during this post-exposure period. It would be an interesting series of experiments to examine how the effects of various post-exposure treatments would impact on this model.) To predict only the deaths during the exposures, HCl and HBr are not included in the equation, since at concentrations normally found in fires, these two gases only have post-exposure effects. To predict deaths that would occur both during and following the exposure, one uses the mathematical model as shown.

The N-Gas Model has been developed into an N-Gas Method. This method reduces the time necessary to evaluate a material and the number of test animals needed for the toxic potency determination. It also indicates whether the toxicity is usual (i.e., the toxicity can be explained by the measured gases) or is unusual (additional gases are needed to explain the toxicity). To measure the toxic potency of a given material with this N-Gas Method, a sample is combusted under the conditions of concern and the gases in the model are measured. Based on the results of the chemical analytical tests and the knowledge of the interactions of the measured gases, an approximate LC_{50} value is predicted. In just two additional tests, six rats are exposed to the smoke from a material sample size estimated to produce an atmosphere equivalent to the approximate LC_{50} level (this can be for within exposure or both within plus post-exposure). The deaths of some percentage of the animals (not 0 and not 100%) indicates that the predicted LC_{50} would be close to the actual calculated LC_{50}. No deaths may indicate an antagonistic interaction of the combustion gases. The deaths of all of the animals may indicate the presence of unknown toxicants or other adverse factors. If more accuracy is needed, then a detailed LC_{50} can be determined.

Another important factor to consider is how well these small-scale bioanalytical approaches predict the toxicological effects observed in real-scale fire tests. Rats were exposed to the gases generated from materials thermally decomposed under various test conditions (the NIST Radiant Panel Method, the Cup Furnace Smoke Toxicity Method and large-scale room tests) *(9, 12-14)*. In most cases, the N-Gas Model was able to predict the deaths correctly. In the case of PVC, the HCl factor was only included in the prediction of the total (within plus post-exposure) deaths. The model correctly predicted the results as long as the HCl was greater than 1000 ppm; therefore, it is possible that HCl concentrations under 1000 ppm may not contribute to lethality even in the post-exposure period. More experiments are necessary to show whether a true toxic threshold for HCl does exist.

Experimental Approaches

Gases. In all tests, chemical analyses were conducted to determine the concentrations of carbon monoxide (CO), carbon dioxide (CO_2), and oxygen (O_2). In tests of nitrogen-containing materials, hydrogen cyanide (HCN) was measured. In some tests, hydrogen chloride (HCl), hydrogen fluoride (HF), hydrogen bromide (HBr) and total nitrogen oxides (NO_x) were also measured to determine if sufficient quantities would be generated to warrant further monitoring. Calibration gases (CO, CO_2, HCN) were commercially supplied in various concentrations in nitrogen. The concentrations of HCN in the commercially supplied cylinders were routinely checked by silver nitrate titration (*15*), since it is known that the concentration of HCN stored under these conditions will decrease with time. Nitric oxide (NO) in nitrogen, a standard reference material, was obtained from the Gas and Particulate Science Division, NIST.

Carbon monoxide and CO_2 were measured continuously during each test by non-dispersive infrared analyzers. Oxygen concentrations were measured continuously with a paramagnetic analyzer. Syringe samples (100 μL) of the chamber atmosphere were analyzed for HCN approximately every three minutes with a gas chromatograph equipped with a thermionic detector (*16*). The concentration of NO_x was measured continuously by a chemiluminescent NO_x analyzer equipped with a molybdenum converter (set at 375°C) and a sampling rate of 25 mL/min. The change from a stainless steel converter to a molybdenum converter prevented interference from HCN. All combustion products and gases (except HCN, NO_x, and the halogen gases) that were removed for chemical analysis were returned to the chamber. The CO, CO_2, O_2 and NO_x data were recorded by an on-line computer every 15 seconds.

The halogen gases, HF, HCl, and HBr, were analyzed by ion chromatography. The combustion products were bubbled into 30 mL impingers containing 25 mL of 5 mM KOH at a rate of approximately 30 mL/min for the 30 minute tests. The flow was monitored every five minutes and averaged over the 30 minute run to determine the amount of gases collected. The resulting solution was analyzed for F^-, Cl^-, and Br^- by the modified method A-106 as described in reference (*17*). In this modified method, the eluent was changed from a 2.5 mM lithium hydroxide solution to a 5 mM KOH solution, a manual injector was used instead of an automatic injector, and a 590 programmable pump was employed instead of the 510 solvent delivery module.

For each test, the reported gas concentrations are the time-integrated average exposure values which were calculated by integrating the area under the instrument response curve and dividing by the exposure time [i.e., (ppm x min)/min or, in the case of O_2, (% x min)/min]. The calculated CO and CO_2 concentrations are accurate to within 100 ppm and 500 ppm, respectively. The calculated HCN concentrations are accurate to 10% of the HCN concentration. The calculated NO_x concentrations are accurate to 10% of the NO_x concentration.

Animals. Fischer 344 male rats, weighing 200-300 grams, were obtained from Taconic Farms (Germantown, NY).* They were allowed to acclimate to our laboratory

*Certain commercial equipment, instruments, materials or companies are identified in this paper to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are the best available for the purpose.

conditions for at least 7 days prior to testing. Animal care and maintenance were performed in accordance with the procedures outlined in the National Institutes of Health's "Guide for the Care and Use of Laboratory Animals." Each rat was housed individually in suspended stainless steel cages and provided with food (Ralston Purina Rat Chow 5012) and water *ad libitum*. Twelve hours of fluorescent lighting per day were provided using an automatic timer. All animals (including the controls) were weighed daily from the day of arrival until the end of the post-exposure observation period.

Determination of Single and Mixed Gas Toxicity. All animal exposures were conducted using the chemical analysis system, and the animal exposure system that were designed for the Cup Furnace and the NIST Radiant Panel Smoke Toxicity Methods (*18,19*).

The exposure chamber is a 200 liter rectangular closed chamber in which all the gases (except the small amounts removed for chemical analysis of HCN, NO_x, HCl, and HBr) are kept for the duration of the test. Six rats are exposed in each test. Each animal is placed in a restrainer and inserted into one of six portholes located along the front of the exposure chamber such that only the heads of the animals are exposed. In the tests conducted to determine various gas LC_{50} values, the desired test concentrations of the gas or gases were generated in the chamber and animal exposures started when the animals were inserted into the portholes. For material LC_{50}'s, the exposure started when the material thermal decomposition was initiated. In most of the studies, animals were exposed for 30 minutes; other exposure times were used to assure the model held for other times as well.

The toxicological endpoint was the LC_{50} values, which were calculated based on the deaths that occurred either during the exposures or the exposure plus at least a 14 day post-exposure observation period. The percentage of animals dying at each gas concentration was plotted to produce a concentration-response curve from which the LC_{50} values and their 95% confidence limits were calculated by the statistical method of Litchfield and Wilcoxon (*20*).

Nitrogen Dioxide and Mixed Gas Toxicity Results and Discussion

NO_2 Toxicity. Deaths from NO_2 in air occur only in the post-exposure period and its LC_{50} following a 30 minute exposure is 200 ppm. The seven experiments to determine this LC_{50} are shown in Figure 1 (open circles) which plots the percent lethality vs. the NO_2 concentration. The concentration which is statistically calculated to correspond to 50% lethality is the LC_{50}.

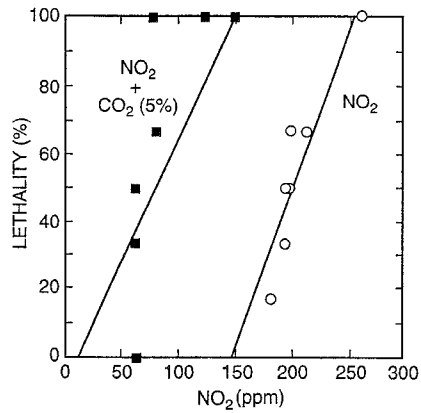
NO_2 plus CO_2. The 30 minute LC_{50} of CO_2 is 47%. Carbon dioxide plus NO_2 showed synergistic toxicological effects (*21*). Figure 1 (solid squares) shows the increase in toxicity (line shifts to the left; LC_{50} value decreases) when the animals are exposed to various concentrations of NO_2 plus 5% CO_2 (*10*). The LC_{50} for NO_2 following a 30 min exposure to NO_2 plus 5% CO_2 is 90 ppm (post-exposure deaths) (i.e., the toxicity of NO_2 doubled).

NO_2 plus CO. Carbon monoxide produces only within-exposure deaths and its 30 min LC_{50} is 6600 ppm (Fig. 2, open circles). In the presence of 200 ppm of NO_2, the within-exposure toxicity of CO doubled (i.e., its 30 minute LC_{50} became 3300 ppm, Fig. 2, solid squares). An exposure of approximately 3400 ppm CO plus various concentrations of NO_2 showed that the presence of CO would also increase the post-exposure toxicity of NO_2. The 30 minute LC_{50} value went from 200 ppm to 150 ppm (Fig. 3, solid squares). We used 3400 ppm of CO as that concentration would not be lethal during the exposure and we would be able to observe the post-exposure effects of CO on NO_2; the LC_{50} of CO (6600 ppm) would have caused deaths of the animals during the 30 minute exposure.

NO_2 plus O_2. The 30 minute LC_{50} of O_2 is 5.4% and the deaths occur primarily during the exposures (Fig. 4, open circles). In the presence of 200 ppm of NO_2, the within-exposure LC_{50} of O_2 and its toxicity increased to 6.7% (Fig. 4, solid squares). In the case of O_2, increased toxicity is indicated by an increase in the value of the LC_{50} since it is more toxic to be adversely affected by a concentration of O_2 ordinarily capable of s-ustaining life. Exposure of the animals to 6.7% O_2 plus various concentrations of NO_2 showed that the NO_2 toxicity doubled (i.e., its LC_{50} value decreased from 200 ppm to 90 ppm) (Fig.5, solid squares).

NO_2 plus HCN. An antagonistic toxicological effect was noted during the experiments on combinations of HCN and NO_2. As mentioned above, the LC_{50} for NO_2 alone is 200 ppm (post-exposure) (Fig. 5). The 30 minute within-exposure LC_{50} for HCN alone is 200 ppm (Fig. 6, open circles). These concentrations of either gas alone is sufficient to cause death of the animals (i.e., 200 ppm HCN or 200 ppm NO_2 would cause 50% of the animals to die either during the 30 min exposure or following the 30 min exposure, respectively). However, in the presence of 200 ppm of NO_2, the within-exposure HCN LC_{50} concentration is 480 ppm or 2.4 times the LC_{50} of HCN alone (Table 1 and Fig. 6, solid squares).

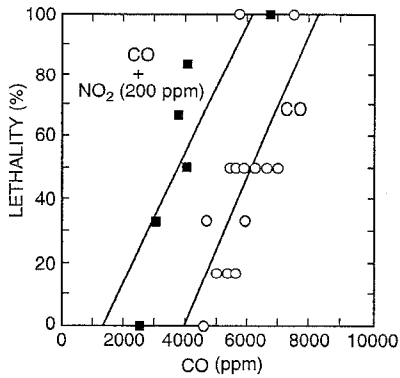
A possible mechanism for this antagonistic effect is as follows: In the presence of H_2O, NO_2 forms nitric acid (HNO_3) and nitrous acid (HNO_2). These two acids are most likely responsible for the lung damage leading to the massive pulmonary edema and subsequent deaths noted following exposure to high concentrations of NO_2. Nitrite ion (NO_2^-) formation occurs in the blood when the nitrous acid dissociates. The nitrite ion oxidizes the ferrous ion in oxyhemoglobin to ferric ion to produce methemoglobin (MetHb) (Equation 2). MetHb is a well-known antidote for CN^- poisoning. MetHb binds cyanide and forms cyanmethemoglobin which prevents cyanide from entering the cells. In the absence of MetHb, free cyanide will enter the cells, react with cytochrome oxidase, prevent the utilization of O_2, and cause cytotoxic hypoxia. If, on the other hand, cyanide is bound to MetHb in the blood, it will not be exerting its cytotoxic effect. Therefore, the mechanism of the antagonistic effect of NO_2 on the toxicity of cyanide is believed to be due to the conversion of oxyhemoglobin [$O_2Hb(Fe^{++})$] to methemoglobin [$MetHb(Fe^{+++})$] in the presence of nitrite [see Equation 2 (*22*) and Figure 7].



Gases	LC_{50} ppm	(95% CL)
NO_2	200	(190-210) PE
CO_2	470,000	(430,000-510,000) WE
$NO_2 + CO_2$	90	(70-120) PE

30 Minute exposures, 5% CO_2, all deaths occurred post-exposure within 24 hours

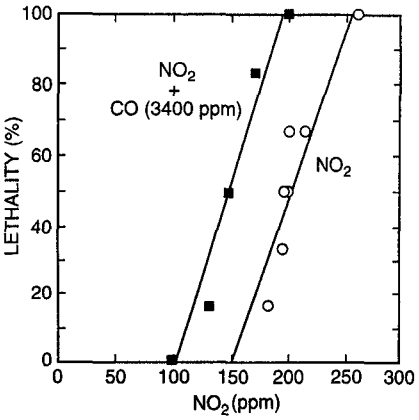
Figure 1. Synergistic effect of CO_2 on NO_2 toxicity. Open circles: NO_2. Solid squares: NO_2 plus 5% CO_2. WE: within exposure; PE: post-exposure. CL: confidence limits.



Gases	LC_{50} ppm	(95% CL)
CO	6600	(6100-7200) WE
NO_2	200	(190-210) PE
$CO + NO_2$	3300	(2800-3900) WE

30 Minute exposures, 200 ppm NO_2, all deaths within exposures

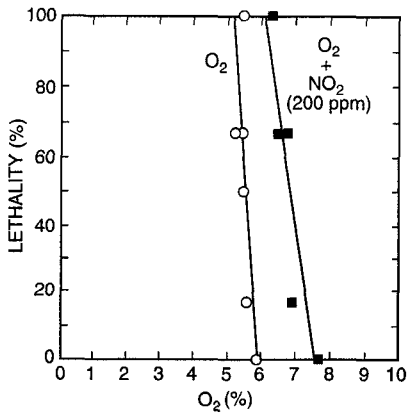
Figure 2. Effect of NO_2 on CO toxicity. Open circles: CO. Solid squares: CO plus 200 ppm NO_2. WE: within exposure; PE: post-exposure. CL: confidence limits.



Gases	LC_{50} ppm	(95% CL)
NO_2	200	(190-210) PE
CO	6600	(6100-7200) WE
NO_2 + CO	150	(140-160) WE + PE

30 Minute exposures, 3300-3600 ppm CO, NO_2 deaths occurred post-exposure, NO_2 + CO deaths occurred within and post-exposure

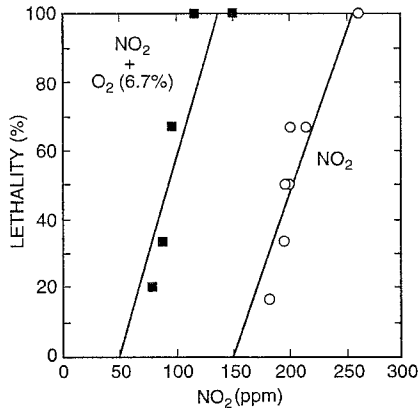
Figure 3. Effect of CO on NO_2 toxicity. Open circles: NO_2. Solid squares: NO_2 plus approximately 3400 ppm CO. WE: within exposure; PE: post-exposure. CL: confidence limits.



Gases	LC_{50}	(95% CL)
O_2	5.4%	(5.5-5.3%) WE
NO_2	200 ppm	(190-210) PE
O_2 + NO_2(200 ppm)	6.7%	(6.8-6.5%) WE

30 Minute exposures, 200 ppm NO_2, only examined within exposure deaths

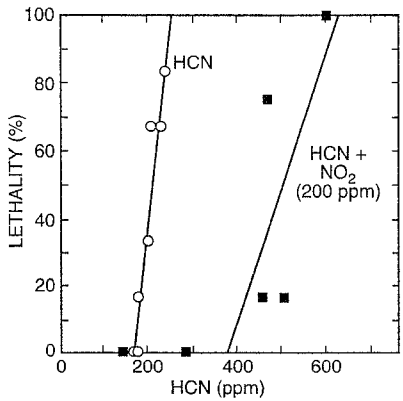
Figure 4. Effect of NO_2 on O_2 toxicity. Open circles: O_2. Solid squares: O_2 plus 200 ppm NO_2. WE: within exposure; PE: post-exposure. CL: confidence limits.



Gases	LC_{50}	(95% CL)
NO_2	200 ppm	(190-210) PE
O_2	5.4%	(5.5-5.3%) WE
$NO_2 + O_2$	90 ppm	(80-100) WE + PE

30 Minute exposures, O_2 at 6.7%, NO_2 deaths occurred post-exposure, $NO_2 + O_2$ deaths occurred within and post-exposure

Figure 5. Effect of O_2 on NO_2 toxicity. Open circles: NO_2. Solid squares: NO_2 plus 6.7% O_2. WE: within exposure; PE: post-exposure. CL: confidence limits.



Gases	LC_{50} ppm	(95% CL)
HCN	200	(190-220) WE
NO_2	200	(190-210) PE
$HCN + NO_2$	480	(390-590) WE

30 Minute exposures, 200 ppm NO_2, all deaths within exposure

Figure 6. Antagonistic Effect of NO_2 on HCN toxicity. Open circles: HCN. Solid squares: HCN plus 200 ppm NO_2. WE: within exposure; PE: post-exposure. CL: confidence limits.

TABLE 1. ANTAGONISTIC EFFECTS OF MIXTURES OF HCN AND NO_2

Gas Concentration[a]		Gas Concentration/LC_{50}[b]				Deaths	
HCN (ppm)	NO_2 (ppm)	HCN WE[c]	HCN WE + PE[d]	NO_2 PE[d]	HCN + NO_2	WE	WE + PE
150	190	0.75	1.00	0.95	1.70[c] 1.95[d]	0/6	3/6
280	200	1.40	1.87	1.00	2.40[c] 2.87[d]	0/6	6/6
460	190	2.30	3.07	0.95	3.25[c] 4.02[d]	1/6	1/6
470	200	2.35	3.13	1.00	3.35[c] 4.13[d]	3/4	4/4
510	190	2.55	3.40	0.95	3.50[c] 4.35[d]	1/6	6/6
600	200	3.00	4.00	1.00	4.00[c] 5.00[d]	6/6	6/6

a: Time-integrated average concentration over the 30 minute exposure [(ppm x min)/min).
b: LC_{50} value (30 min exposure): HCN (WE) = 200 ppm; HCN (WE + PE) = 150 ppm; NO_2 (PE) = 200 ppm.
c: WE: Within the 30 minute exposure.
d: WE + PE: Within the 30 minute exposure plus the post-exposure observation period.

$$2H^+ + 3NO_2^- + 2O_2Hb(Fe^{++}) =$$

$$2MetHb(Fe^{+++}) + 3NO_3^- + H_2O \qquad (2)$$

Tertiary Mixtures of NO_2, CO_2, and HCN. Since the binary gas mixture studies indicated that NO_2 plus CO_2 showed synergistic toxic effects and NO_2 plus HCN showed antagonistic toxic effects, it was of interest to examine combinations of NO_2, CO_2, and HCN. In this series of experiments, the concentrations of HCN were varied from almost 2 to 2.7 times its LC_{50} value (200 ppm). The concentrations of NO_2 were approximately equal to one LC_{50} value (200 ppm) if the animals were exposed to NO_2 alone and approximately 1/2 the LC_{50} (90 ppm or twice as toxic) if the animals were exposed to NO_2 plus CO_2; the concentrations of CO_2 were maintained at approximately 5%; and the O_2 levels were kept above 18.9 %. The results of these experiments are shown in Table 2.

Earlier work (*3,10*) indicated that the presence of 5% CO_2 with either HCN or NO_2 produced a more toxic environment than would occur with either gas alone. The antagonistic effects of NO_2 on HCN shown in Table 1 indicate that the presence of one LC_{50} concentration of NO_2 (~ 200 ppm) will protect the animals from the toxic effects of HCN during the 30 minute exposures, but not from the post-exposure effects of the combined HCN and NO_2. However, results in Table 2 indicate that CO_2 does not make the situation worse, but rather provides protection even during the post-exposure period. In each of the six experiments shown in Table 2, some or all of the animals lived during the test and in 4 tests, some of the animals lived through the post-exposure period even though column 12 of Table 2 shows that the animals were exposed to combined levels of HCN, NO_2 and CO_2 that would be equivalent to 4.7 to 5.5 times the lethal concentrations of these gases. One possible reason that CO_2 seems to provide an additional degree of protection is that NO_2 in the presence of 5% CO_2 produces 4 times more MetHb than does NO_2 alone (Fig. 8) (*10*).

Mixtures of CO, CO_2, NO_2, O_2, and HCN. The initial design of these experiments was to look for additivity of the CO/CO_2, HCN, and NO_2 factors keeping each at about 1/3 of its toxic level, while keeping the O_2 concentration above 19%. When these initial experiments produced no deaths, we started to increase the concentrations of CO up to 1/3 of the LC_{50} of CO alone (6600 ppm), HCN was increased to 1.3 or 1.75 times its LC_{50} depending on whether the within-exposure LC_{50} (200 ppm) or the within- and post-exposure LC_{50} (150 ppm) is being considered, and NO_2 was increased up to a full LC_{50} value (200 ppm). The results indicated that just adding a NO_2 factor (e.g., $[NO_2]/LC_{50}$ NO_2) to Equation 1 would not predict the effect on the animals. A new mathematical model was developed and is shown as Equation 3. In this model, the differences between the within-exposure predictability and the within-exposure and post-exposure predictability is: (1) the LC_{50} value used for HCN is 200 ppm for within-exposure or 150 ppm for within-exposure and post-exposure and (2) the HCl and HBr factors are not used to predict the within-exposure lethality, only the within-exposure and post-exposure

TABLE 2. TERTIARY MIXTURES OF NO_2, CO_2, AND HCN

Gas Concentrations[a]				Gas Concentration/LC_{50}[b]								Deaths	
HCN (ppm) (1)	NO_2 (ppm) (2)	CO_2 (ppm) (3)	O_2 (%) (4)	HCN WE[c] (5)	HCN WE + PE[d] (6)	NO_2 PE (7)	$NO_2 + CO_2$ PE (8)	HCN[c] + NO_2 (9)	HCN[d] + NO_2 (10)	HCN[c] + ($NO_2 + CO_2$) (11)	HCN[d] + ($NO_2 + CO_2$) (12)	WE[c]	WE + PE[d]
380	200	51400	19	1.91	2.55	0.98	2.18	2.89	3.53	4.09	4.72	2/6	3/6
410	200	51300	19	2.07	2.75	0.98	2.18	3.05	3.73	4.24	4.93	1/6	3/6
430	200	49700	19	2.17	2.89	0.98	2.17	3.14	3.86	4.33	5.05	2/6	6/6
460	190	53300	19	2.29	3.05	0.97	2.16	3.26	4.02	4.44	5.20	2/6	4/6
500	190	54300	19	2.51	3.35	0.95	2.12	3.47	4.30	4.63	5.47	0/6	3/6
550	190	50400	19	2.74	3.65	0.97	2.14	3.70	4.61	4.88	5.79	5/6	6/6

a: Columns 1 - 4: Time-integrated average concentration over the 30 minute exposure (ppm x min/min).
b: LC_{50} value (30 min exposure): HCN (WE) = 200 ppm; HCN (WE + PE) = 150 ppm; NO_2 (PE) = 200 ppm; NO_2 in the presence of 5% CO_2 (PE) = 90 ppm.
c: WE: Within the 30 minute exposure.
d: WE + PE: Within exposure + Post-exposure observation period.
Column 5 = Column 1/200 ppm [i.e., LC_{50} HCN (WE)].
Column 6 = Column 1/150 ppm [i.e., LC_{50} HCN (WE + PE)].
Column 7 = Column 2/200 ppm [i.e., LC_{50} NO_2(PE)].
Column 8 = Column 2/90 ppm [i.e., LC_{50} NO_2 in presence of 5% CO_2 (PE)].
Column 9 = Columns 5 + 7.
Column 10 = Columns 6 + 7.
Column 11 = Columns 5 + 8.
Column 12 = Columns 6 + 8.

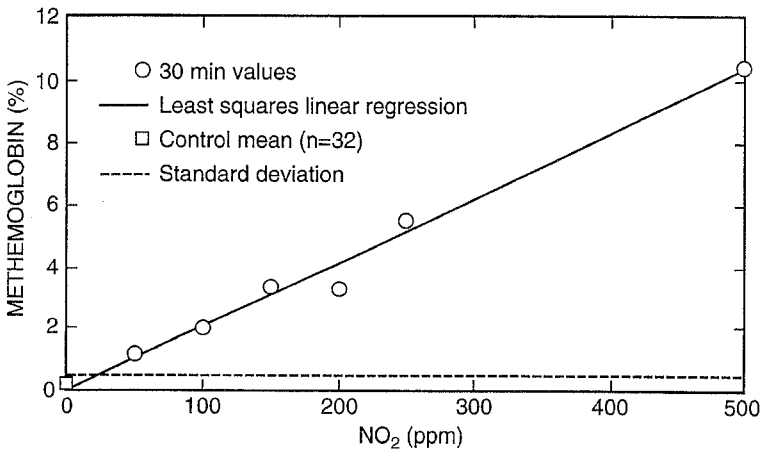


Figure 7. Methemoglobin values from 30 minute exposures to various concentrations of NO_2. Modified and reprinted from reference 10 with permission of the author.

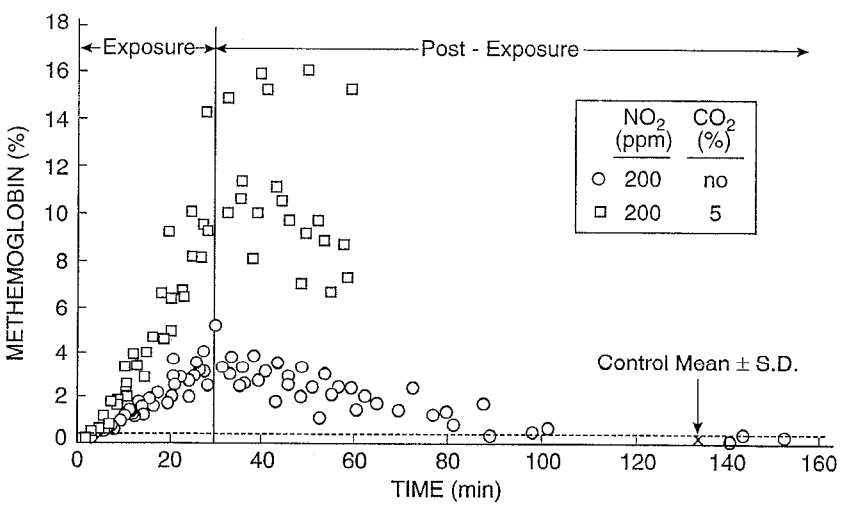


Figure 8. Methemoglobin generation during and following exposure to NO_2 alone (open circles) or NO_2 plus 5% CO_2 (open squares). Control mean ± the standard deviation (S.D.) was 0.2 ± 0.2 (n = 32 animals). Modified and reprinted from reference 10 with permission of the author.

lethality. According to Equation 3, animal deaths will start to occur when the N-Gas Value is above 0.8 and 100% of the animals will die when the value is above 1.3. The experimental results supporting this model are presented in Table 3. Results in Table 3 indicate that in those cases where the values were above 0.8 and no deaths occurred, the animals were severely incapacitated (close to death) as demonstrated by no righting reflex or eye reflex.

The N-Gas Model Including NO_2.

$$N\text{-}Gas\ Value = \frac{m\,[CO]}{[CO_2] - b} + \frac{21 - [O_2]}{21 - LC_{50}(O_2)} + \left(\frac{[HCN]}{LC_{50}(HCN)} \times \frac{0.4\,[NO_2]}{LC_{50}(NO_2)}\right) + 0.4\left(\frac{[NO_2]}{LC_{50}(NO_2)}\right) + \frac{[HCl]}{LC_{50}(HCl)} + \frac{[HBr]}{LC_{50}(Hbr)} \qquad (3)$$

For an explanation of these terms, see the paragraph following Equation 1. Equation 3 should be used to predict the within-exposure plus post-exposure lethal toxicity of mixtures of CO, CO_2, HCN, reduced O_2, NO_2, HCl, and HBr. The LC_{50} values will be the same as those given for Equation 1 using 150 ppm for HCN and 200 ppm for NO_2. If one wishes to predict the deaths that will occur only during the exposure, the LC_{50} value used for HCN should be 200 ppm and the HCl and HBr factors should not be included. To predict the lethal toxicity of atmospheres that do not include NO_2, Equation 1 is to be used.

Conclusions

In the binary gas studies, NO_2, a toxic gas which exerts its lethal effect following an exposure, increased the toxicity of all the tested within-exposure toxic gases except HCN. The reverse was also seen - i.e., the post-exposure toxic effects of NO_2 were greater if the animals had also been exposed to CO, CO_2, or reduced O_2. The exception to these results occurred when the animals were exposed to NO_2 plus HCN, a combination which produced an antagonistic toxicological effect. The explanation for this antagonistic effect is believed to be due to the production of methemoglobin (a cyanide antidote) by nitrite ions formed from the dissociation of nitrous acid in the blood. The nitrous acid was generated by the reaction of NO_2 and H_2O in the lung.

Although the binary combinations of NO_2 and CO_2 showed that the toxicity of NO_2 doubled, the tertiary gas combination studies with HCN, NO_2, and CO_2 indicated that CO_2 did not increase the toxicity of the mixture, but may have further increased the protective effect of the NO_2. This increased protective effect of CO_2 is probably due to

TABLE 3. Mixtures of CO, CO_2, NO_2, O_2, and HCN.

Gas Concentration					N-Gas Value WE	N-Gas Value WE + PE	Deaths WE	Deaths WE + PE	Incapacitation (% of survivors)
CO (ppm)	CO_2 (ppm)	HCN (ppm)	O_2 (%)	NO_2 (ppm)	Equation 3	Equation 3			
1280	51800	50	19.4	70	0.6	0.62	0/6	0/6	0
1310	51800	80	19.4	100	0.71	0.73	0/5	0/5	0
2030	46800	50	19.6	90	0.8	0.81	1/6	2/6	80
2060	45800	50	19.7	100	0.82	0.84	2/6	4/6	100
2180	51000	70	19.5	70	0.83	0.85	0/6	0/6	66.7
2160	50800	50	19.4	90	0.88	0.9	0/6	1/6	16.7
2210	50900	50	19.4	110	0.93	0.95	0/6	2/6	16.7
1900	49700	160	19.4	190	1.26	1.36	4/6	6/6	100
1930	50500	190	19.3	200	1.36	1.46	4/6	6/6	100
1980	53700	210	19.1	200	1.42	1.55	6/6	6/6	N/A
2210	53200	260	19.2	200	1.58	1.75	6/6	6/6	N/A

WE: Within exposure
PE: Post-exposure
Incap: Incapacitation

the greater amount of MetHb produced by the combination of NO_2 and CO_2 than by NO_2 alone as shown by earlier work in our laboratory. Earlier work in this laboratory showed that more methemoglobin is produced by the combination of NO_2 and CO_2 than by NO_2 alone.

A new N-Gas Model was needed to move from the 6-gas model which includes CO, CO_2, HCN, reduced O_2 concentrations, HCl and HBr to a 7-gas model which also includes NO_2. Validation studies looking at a series of materials and products under conditions ranging from laboratory bench-scale to full-scale room burns indicated that, in all cases, the 6-Gas Model was able to predict the deaths correctly. In the case of PVC, the HCl factor was only included in the prediction of the post-exposure (not within-exposure) deaths and preliminary data showed that concentrations under 1000 ppm may not have any observable effect even in the post-exposure period. More experiments are necessary to show whether HCl has a toxic threshold. The 7-Gas model works when the animals are exposed to various concentrations of the tested gases; studies need to be done to ensure that the 7-Gas Model predicts the outcome when nitrogen-containing materials and products are thermally decomposed.

Caution: The values given for use in equations 1 and 3 are dependent on the test protocol, on the source of test animals, and on the rat strain. It is important to verify the above values whenever different conditions prevail and if necessary, to determine the values that would be applicable under the new conditions.

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